Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Assessment of antibacterial drug residues in milk for consumption in Kosovo

Adem Rama [a,*], *Lorena Lucatello* [b], *Cristiana Benetti* [c], *Guglielmo Galina* [b], *Drita Bajraktari* [d]

[a] *Faculty of Agriculture and Veterinary, University of Prishtina, Prishtina, Kosovo*
[b] *Department of Comparative Biomedicine and Food Science, Legnaro, Padova, Italy*
[c] *Food Safety Department, Chemistry Laboratory Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy*
[d] *Food and Veterinary Agency, Prishtina, Kosovo*

ARTICLE INFO

Article history:
Received 26 April 2016
Received in revised form
25 June 2016
Accepted 22 July 2016
Available online 5 November 2016

Keywords:
antibacterial residues
detection method
Kosovo
milk
public health

ABSTRACT

The objective of this study was to assess the occurrence of drug residues in the raw milk collected from individual farms and milk collection points during 2009–2010 in six different major regions of Kosovo (Prishtinë, Gjilan, Mitrovicë, Pejë, Gjakovë, Prizren). In the present study, a total of 1734 raw milk samples were collected, and qualitatively screened with two different tests, the Delvotest SP assay and an enzyme-linked receptor-binding assay (SNAP). Overall, 106 (6.11%) out of 1734 samples examined with Delvotest SP contained possible drug residues (5.12% and 7.51% of samples from 2009 and 2010, respectively). All suspect samples were further analyzed by three distinct enzyme-linked receptor-binding assays specific for β-lactams (new β-lactam test), tetracyclines (SNAP tetracycline test), and sulfonamides (SNAP sulfamethazine test). Only the new SNAP β-lactam test detected residues in 40 out of 52 samples in 2009 and 54 out of 54 suspect samples in 2010. A confirmatory method based on liquid chromatography-tandem mass spectrometry was used to confirm the presence of β-lactam drug residues in samples detected by the enzyme-linked receptor-binding assay. Amoxicillin, penicillin G, and cloxacillin were the most frequently detected residues and were in a concentration range between 2.1 μg/kg and 1973 μg/kg. Seventeen of the positive samples exceeded the maximum residue levels for one or more β-lactam drug. The highest number of positive milk samples came from the Pejë Region (58.8%) and Gjakovë Region (23.5%), and the lowest number of positive samples originated from Gjilan (5.88%), with no positive samples detected in two regions, Mitrovicë and Prizren.

* *Corresponding author*. University of Prishtina Agriculture and Veterinary Faculty, Bulevard "Bill Clinton" N.N., 10000, Prishtina, Kosovo. Fax: +381 (0)38603102.
E-mail address: adem.rama@uni-pr.edu (A. Rama).

http://dx.doi.org/10.1016/j.jfda.2016.07.007

1. Introduction

Antibiotic residues in milk are of great concern to dairy farmers, milk processors, regulatory agencies, and consumers. In lactating cows, antimicrobial agents are used mostly for the therapy of mastitis but are used to treat other diseases as well. Today, antimicrobial drugs are used to control, prevent, and treat infection, and to enhance animal growth and feed efficiency [1]. Currently, approximately 80% of all food-producing animals receive medication for part or most of their lives [2]. The most likely cause of violative drug residues is the failure to observe proscribed withdrawal times [3–6]. The presence of antimicrobial residues in milk can engender drug hypersensitivity reactions in milk consumers, manifested as dermal reactions, asthma, or anaphylactic shock [7–12]. Antimicrobial drugs can also interfere with the manufacture of dairy products, decrease acid and flavor production associated with butter manufacture, reduce the curdling of milk, and cause improper ripening of cheeses [13,14]. Finally, the use of antibiotics can give rise to an increase in antibiotic resistance of pathogenic bacteria and contribute to a global health crisis [15,16].

In many countries, governmental authorities have established monitoring programs to determine the antibiotic levels in food and set a maximum residue level (MRL) for these drugs. In the European Union, veterinary drug residue monitoring is enforced according to the requirements set down in Council directive 96/23/EC [18] and Commission Decision 97/747/EC [19], and the MRLs were fixed according to Regulation 470/2009/CE [20] and Regulation 37/2010/UE [21]. The Kosovo program of monitoring residues in live animals and animal products has been in place since 2005. Various analytical methods in detecting antibiotic residues in milk have been reported in the literature [17,22]. Microbiological growth inhibition, enzyme-linked immunosorbent assays, and chromatographic methods are the most commonly used [23,24].

Kosovo's dairy sector is one of the key sectors in the development of agriculture and continues to recover after the war in 1999, when at least half of livestock production was depleted. Milk production is widespread throughout Kosovo, with more than 25 dairy processing companies in operation [25]. These dairies produce some 381,896 tons of milk annually, and 58,563.45 tons are imported. The market value of locally produced milk was €35,934,158 and from imports it was €32,463,988 [26]. In Kosovo, there is currently no monitoring of drug residues in milk. Hence, there are no data concerning the presence of antibiotic residues in milk produced and marketed in Kosovo. The present study was therefore designed to assess the presence of antimicrobial drug residues in raw milk marketed at different regions of Kosovo.

2. Methods

2.1. Samples

A total of 1734 milk samples from individual farms and milk collection points were collected over a 2-year period (April–October 2009 and February–November 2010) from six major regions of Kosovo (Prishtinë, Gjilan, Mitrovicë, Pejë, Gjakovë, Prizren). In 2009, a total of 1015 milk samples were collected, 826 samples from milk collection points and 189 samples from individual farms. In 2010, in total of 719 milk samples were collected, 635 samples from milk collection points and 84 samples from individual farms. All milk samples were stored at 4°C until analysis. For additional investigations, drug-positive milk samples were stored at −20°C for 3 weeks.

2.2. Screening methods

Antimicrobial drug screening tests were performed at the Kosovo Food and Veterinary Agency in Prishtina, Kosovo. The screening tests used were the Delvotest SP assay supplied by DSM (DSM Food Specialities, Dairy Ingredients, Delft, The Netherlands), and enzyme-linked receptor-binding assays (SNAP tests) provided by IDEXX Lab. Inc. (Westbrook, ME, USA). All drug-positive samples detected by the Delvotest SP were checked with enzyme-linked receptor-binding assays specific for β-lactams, tetracycline, and sulfonamides. Positive samples confirmed by SNAP test were further quantitatively analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2.2.1. Reagents and standard solutions for screening tests

Penicillin G (PNG) potassium salt and sulfamethazine were obtained from Fluka (St. Louis, MO, USA), Tetracycline hydrochloride was obtained from Sigma (St. Louis, MO, USA). For the preparation of negative control, drug-free milk from cows that had not been treated with an antibiotic for at least 30 days was collected. The milk was collected from the experimental farm of the Agriculture and Veterinary Faculty (Prishtina, Kosovo).

A stock solution of PNG potassium was prepared in a 100-mL volumetric flask, adding 11.17 mg penicillin and distilled water to the target volume. From this solution, 1 mL (100 μg penicillin/mL) was diluted 100-fold with distilled water (i.e., to a final concentration of 1 μg penicillin/mL).

For preparation of drug-spiked milk samples, drug concentrations of $\geq$ 40 μg/L of milk were prepared by adding the appropriate amount of stock solution directly to milk samples. The equivalent volume of milk was removed prior to adding the appropriate volume of stock solution. The amount added was always $<$ 0.5% of the total volume. Penicillin-G potassium was present at a final concentration of 4 μg/L for the positive control sample, whereas tetracycline and sulfamethazine were added to milk to achieve final concentrations of 60 μg/L and 100 μg/L, respectively.

2.2.2. Delvotest SP microbial test

The qualitative analysis of PNG residues in milk was performed using the Delvotest SP assay as described by Suhren and Beukers [27]. This method is based on the susceptibilities of bacteria to different antibiotics. The method was carried out according to the instructions by the manufacturer.

2.2.3. Enzyme-linked receptor-binding assays (SNAP tests)

Positive samples found by Delvotest SP were subjected to further testing with enzyme-linked receptor-binding assays (SNAP tests). The New SNAP Beta-lactam Test Kit, SNAP